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SEROPREVALENCE OF *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM* IN URBAN AND RURAL DOGS FROM SOUTHWESTERN NIGERIA

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Abstract

Background: *Toxoplasma gondii* and *Neospora caninum* are protozoans infecting a wide range of mammals; the etiologic agents of Toxoplasmosis and Neosporosis respectively, This study investigated the prevalence of antibodies to *Toxoplasma gondii* and *Neospora caninum* in dogs from southwestern Nigeria.
Materials and Methods: A total of 233 serum samples were obtained from both urban and rural areas of Oyo state, Nigeria and tested by the western blotting technique for specific IgG to *T. gondii* and *N. caninum*.
Results: The seroprevalence obtained for *T. gondii* was 19% (44/233) and 2.1% (5/233) for *N. caninum* in the dogs examined. Overall, the prevalence of antibodies to *T. gondii* was more significant than for *N. caninum* ($P< 0.0001$). *T. gondii* infection was also found to be more significant in roaming than in caged dogs ($P< 0.05$). There was no significant association of other factors (age, breed, history of rabies vaccination, use of dog and the level of education of owner) with the prevalence of *T. gondii* and *N. caninum* infection.
Conclusion: This report revealed that *T. gondii* infection is more common than *N. caninum* infection in Nigerian dogs, it suggests that improper housing and feeding of dogs could increase the risk of exposure to *T. gondii* infection. This is the first study to investigate the seroprevalence of *N. caninum* antibodies in Nigerian dogs and *T. gondii* antibodies in dogs in southwestern Nigeria.

key words: *Toxoplasma gondii*, *Neospora caninum*, Dogs, Protozoan, Zoonoses, Nigeria.

Introduction

Toxoplasma gondii and *Neospora caninum*, the etiologic agents of Toxoplasmosis and Neosporosis respectively, are morphologically similar apicomplexan protozoans infecting a wide range of mammals (Dubey and Lindsay, 1996).

Toxoplasma gondii infection is an important parasitic zoonosis that infects a variety of hosts (including humans, domestic animals and birds) mainly through the ingestion of undercooked meat containing tissue cysts and water or food contaminated by oocysts shed by cats (the primary host) into the environment (Freckle et al., 1975; Dubey, 2010; Pereira et al., 2010). Infection from an infected mother to the fetus vertically has also been reported in mammals (Dubey, 2010; Tenter et al., 2000). Dogs infected with *T. gondii* could transmit the oocyst to humans mechanically (Lindsay et al., 1997; Frenkel et al., 2003). Outdoor dogs and other free-living animals that scavenge from the ground are used as indirect indicators of environmental contamination by *T. gondii* oocysts (Dubey and Frenkel, 1998; Meireles et al., 2004; Salb et al., 2008).

Neospora caninum, like *T. gondii,* also causes disease in a wide range of animals, most significantly in cattle where it causes abortion and other reproductive losses (Dubey and Lindsay, 1996). However, in contrast to *T. gondii*, it has not been known to infect humans. *N. caninum* may be transmitted via consumption of feed or water contaminated with the oocysts of the parasite or by vertical transmission of the tachyzoite stage from dam to foetus during pregnancy (Buxton et al., 2002). Dogs and coyotes have so far been described as the definitive hosts (McAllister et al., 1998; Gondim et al., 2004).

The seroprevalence of *T. gondii* and *N. caninum* infections has been extensively studied worldwide, but there is paucity of information on these infections in Nigerian dogs. There has been no report on the occurrence of *N. caninum* antibodies in dogs in Nigeria and the only available report on *Toxoplasma gondii* in dogs in Nigeria is that by Kamani et al. (2010) obtained from a study carried out on dogs in northern Nigeria. Meanwhile, there is currently an increase in the demand for exotic breeds of dogs as pets and security dogs in the urban areas and the population of local dogs is also soaring in the rural areas where dogs are used mainly for hunting. This study is therefore aimed at investigating the exposure of dogs to *T. gondii* and *N. caninum* in the rural and urban areas of southwestern Nigeria.

Materials and Methods

Location and sampling

The study was conducted in Oyo (latitude 7° N to 9° N, longitude 2.8° E to longitude 4.5° E) and Ogun states (latitude 6.2° N to 7.8° N, longitude 3° E to longitude 5° E), an area that is approximately 44,863 square kilometers within the rainforest area of West Africa. Serum samples were randomly collected from a total of 233 dogs (49 exotic and 184 local) in both the urban and rural communities. Obtained sera were shipped to

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the Friedrich Loeffler Institute, Germany, for analysis. Data on breed, age, sex, use of dog (companion or hunting), housing, vaccination status and educational level of owner were obtained and matched.

Detection of *T. gondii* and *N. caninum* antibodies

The prevalence of anti-*T. gondii* and *N. caninum* antibodies in obtained sera was determined by the Western blott technique using antigens (total and purified) of *T. gondii* and *N. caninum* respectively as described by Damriyasa et al. (2004) and Azevedo (2010).

Data Analysis

Data were analyzed by Fisher's exact test using Graphpad Prism Software (GraphPad Software Inc. La Jolla, CA). Statistically significant difference and associations were observed if the p-value was less than 0.05.

Results

The overall seroprevalence obtained for *T. gondii* was 19% (44/233) and 2.1% (5/233) for *N. caninum* (Table 1). Seropositivity for *T. gondii* was higher in local dogs [20% (37/184)] than in the exotic breeds of dogs [14% (7/49)]. Antibodies to *N. caninum* were only found in 2.7% (5/184) of local dogs and none in the exotic breeds of dog. Antibodies to both *T. gondii* and *N. caninum* were only found in 2.2% (4/184) of the local dogs and none in exotic breeds. Overall, the prevalence of antibodies to *T. gondii* was significantly different from that of *N. caninum* (p< 0.0001). *T. gondii* infection was more significant in roaming than in caged dogs (p=0.001) with the roaming dogs having about twelve times the odds of getting infected than those housed in cages (Tables 2 and 3). In this study, we found no significant association between factors like age, breed, history of rabies vaccination, use of dog and the level of education of owner and the seroprevalence of *T. gondii and N. caninum* in the dog population examined.

Table1: Seroprevalence of antibodies to *T. gondii* and *N. caninum* in dogs in southwestern Nigeria

	No. Tested	No. Positive	Percentage	Odds ratio (95% C.I.)	P-value
T. gondii	233	44	19	11.21 (4.354 - 28.86)	P<0.0001
N. caninum	233	5	2.1	0.08921 (0.03465 - 0.2297)	
T. gondii & N. caninum	233	4	1.7		

Discussion

The overall prevalence obtained for *T. gondii* antibodies in dogs in our study was lower than 25% reported by Kamani et al. (2010) in Northern Nigeria while values ranging from 0.26% (Lu et al., 2009) to 21.6% (Duan et al., 2012) had been reported in China and seroprevalence as high as 89% was obtained from a study in Brazil (Lindsay et al., 1997). For *N. caninum*, the seroprevalence obtained in our study was higher than the report from Sweden where Björkman and Uggla (1994) reported a prevalence of 0.5% in Swedish domestic dogs. Interestingly, the present study found no antibodies to *N. caninum* in domestic (companion) dogs but only in outdoor dogs used for hunting. The variation in the seroprevalence of *T. gondii* and *N. caninum* in dogs from one region to the other has been suggested to be associated with ecological and geographical factors, and welfare conditions of the dogs (Dubey and Lindsay, 1996; Duan et al., 2012). The sensitivity and specificity of detection tests utilized has also been suggested as a factor that influences the prevalence result from different parts of the world (Dubey, 2010).

Our study showed that dogs in the studied area are exposed to both *T. gondii* and *N. caninum* infection although exposure to *T. gondii* was found to be more significant than to *N. caninum*. This is not surprising as *T. gondii* is known to be ubiquitous and endemic in most areas of the world with the capability of infecting an unusually wide range of hosts (Tenter et al., 2000). Our findings also showed that there was a significant association between the presence of *T. gondii* antibodies and roaming (or free-living) dogs than those that live in the cage. The higher exposure of roaming dogs could be attributed to unrestricted access to food and water contaminated with *T. gondii* oocysts shed by cats. Roaming dogs are also known to prey on rodents or chickens that are intermediate hosts of *T. gondii*, while caged dogs on the other hand are well cared for and fed with food from their owners. A similar reason could account for the detection of *N. caninum* antibodies in roaming dogs and their absence in caged dogs. The prevalence of *T. gondii* infection in roaming dogs has been suggested as a predictor for risk of human exposure since both dogs and humans are in contact with the same environment (Tenter et al., 2000; Meireles et al., 2004) while seropositivity in dogs has been found to be a major risk factor for *N. caninum* infection in cattle (Dubey et al., 2007).

Furthermore, we report here the detection of higher antibody titre to *T. gondii* in local than in exotic dog breeds as well as the occurrence of both *N. caninum* and *T. gondii* antibodies only in local dogs. The reasons for this finding could also be related to management practices. Most local dogs are left to roam around and fend for themselves; while the exotic breeds are mostly housed in kennels and well cared for because they are highly

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priced and valued. For example, most exotic breeds of dogs in Nigeria are reared as security dogs and also for commercial breeding purposes.

It is also worth noting that of all the exotic dog breeds studied, *T. gondii* antibodies were only found in the Alsatian breed. This is not a surprise since the Alsatian is the cheapest and the most common of all the exotic breed of dogs in Nigeria. Hence, this finding suggests that the value and care of dogs could influence the risk of exposure to *T. gondii* infection.

Table 2: Associations of factors with seroprevalence of antibodies to *T. gondii* in Dogs in southwestern Nigeria

Characteristics	Group		No. Tested	No. Positive for T. *gondii* (%)	OR (95%CI)	p-value
BREED	Local		184	37(20)	1.151 (0.628 - 3.633)	0.4167
	Exotic		49	7 (14)		
		Alsatian	38	7(18)		
		Boerbull	3	0		
		Rigdeback	1	0		
		Terrier	1	0		
		Rottwellier	*6*	*0*		
SEX	Female		132	26 (20)	1.131 (0.580 - 2.202)	0.739
	Male		101	18 (17.8)		
AGE	Adult (> 6 months)		195	38 (19)	1.291 (0.503 - 3.309)	0.821
	Young (< 6 months)		38	6 (16)		
TYPES	Hunting		156	32 (21)	1.398 (0.674 - 2.896)	0.477
	Companion		77	12 (16)		
HOUSING	Roaming		189	43 (23)	12.66 (1.693 - 94.71)	0.001*
	Caged		44	1 (2.3)		
LOCATION	Rural		157	33 (21)	1.573 (0.746 - 3.315)	0.285
	Urban		76	11 (14)		
RABIES VACCINATION	NO		195	38 (19)	1.291 (0.503 - 3.309)	0.821
	YES		38	6 (16)		
EDUCATED OWNERS	NO		169	34 (20)	1.360 (0.628 - 2.945)	0.574
	YES		64	10 (16)		

Table 3: Associations of factors with seroprevalence of antibodies to *N. caninum* in Dogs in southwestern Nigeria

Characteristics	Group		No. Tested	No. Positive for T. *gondii* (%)	OR (95%CI)	p-value
BREED	Local		184	5(2.7)	3.033 (0.165 - 55.84)	0.5868
	Exotic		49	0		
		Alsatian	38	0		
		Boerbull	3	0		
		Rigdeback	1	0		
		Terrier	1	0		
		Rottwellier	6	0		
SEX	Female		132	4 (3)	0.320 (0.035 - 2.909)	0.392
	Male		101	1 (0.99)		
AGE	Adult (> 6 months)		195	5 (2.5)	2.223 (0.120 - 47.1)	1.000
	Young (< 6 months)		38	0		
TYPES	Hunting		156	5 (3.2)	5.627 (0.307- 103.2)	0.174
	Companion		77	0		
HOUSING	Roaming		189	5 (2.6)	2.653 (0.144 - 48.91)	0.587
	Caged		44	0		
LOCATION	Rural		157	5 (3.2)	5.518 (0.301 - 101.2)	0.176
	Urban		76	0		
RABIES VACCINATION	NO		195	5 (2.5)	2.223 (0.120 - 47.1)	1.000
	YES		38	0		
EDUCATED OWNERS	NO		169	5 (3)	4.313 (0.235 - 79.18)	0.326
	YES		64	0		

Although our study showed that the age, sex, use of dogs (either for hunting or as companion animals), their location (rural or urban areas), history of rabies vaccination and the level of education of owners were not significantly associated with *T. gondii* and *N. caninum* infections, the detection of more antibodies to *T. gondii* in hunting and rural dogs than in samples from companion and urban dogs suggest the likelihood of greater exposure to infection sources like faeces of feral cats in the rural than the urban areas. We also observed that higher level of antibodies to *T. gondii* was detected in dogs without history of rabies vaccination and those owned by non-educated individuals. This finding suggests that the vaccination status of dogs as well as owner's educational status could be indirect indicators of risk of exposure to *T. gondii* infection.

In conclusion, the present study showed that *T. gondii* infection is more common than *N. caninum* infection in dogs in Nigeria, and dogs that are allowed to roam and fend for themselves are more at risk of getting infected with both parasites. Dog owners should ensure proper confinement and care of dogs to restrict access to sources of infection in the environment. Health education programs for animal owners/handlers and enforcement of capturing of stray pets should also be carried out as preventive measure against toxoplasmosis and neosporosis. This study is the first to describe the presence of antibodies to *T. gondii* in dogs in southwestern Nigeria and also present the first evidence of *N. caninum* antibodies in Nigerian dogs.

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Conflict of interest: All authors declare that they or their institutions have no financial and personal relationship with other people or organizations that could inappropriately influence their work.

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